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Original article

Bioactive compounds, pigments, antioxidant activity and antimicrobial activity of yellow prickly pear peels

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Summary The main goal of this research was to study the effects of pressure, extraction time and ethanol concentration on antioxidant activity, total phenolics, flavonoids, anthocyanins, carotenoids and betalains compounds extraction from yellow prickly pear peels. A Box–Behnken design and Response Surface Methodology were used to evaluate the effects and estimate the optimum extraction conditions. Antimicrobial activity was evaluated against *Escherichia coli* and *Listeria innocua*. Ethanol concentration was the variable that showed the highest effect on extraction yields but high-pressure increased extraction yields between 6% and 17%. Models showed good fitting and adequacy to the experimental data and the high correlation of models indicated that it can be employed to optimise extraction conditions. The experimental and predicted values differed <10% and the extracts inhibited the growth of both bacteria. High-pressure could be a promising extraction process to improve extraction of bioactives from prickly pear peels.

Keywords Antibacterial activity, antioxidant activity, bioactive compounds, high-pressure extraction, prickly pear peels.

Introduction

Prickly pear (*Opuntia* spp.) fruit is eaten raw or processed. The peel of commercially ripe fruit accounts for 33% to 55%, while the pulp accounts for 45% to 67% and the seeds, contained in the pulp, accounts for 2% to 10%. Processing generates high amounts of fruit residues that may create value in the entire chain-production since prickly pear peels are excellent sources of bioactive compounds, such as betalains, phenolic compounds and flavonoids (Barba *et al.*, 2017). Betalains can be used as a natural food colourant, but also have antioxidant, anti-inflammatory, anti-microbiological, anti-cancer and anti-lipidaemic properties as well as phenolic compounds, playing an important role in the prevention of some diseases (Aragona *et al.*, 2018).

Bioactive compounds can be extracted using traditional methods but usually require extended extraction times, consumption of large volumes of solvents, the extraction yields and selectivity are low and the high temperature applied can degrade/volatilise important compounds (Mojzer *et al.*, 2016). Attempting to overcome these limitations, high-pressure has been studied as extraction method since it is environmental friendly, reduce the synthetic and organic chemicals used, require less extraction times, achieves better yields and the extracts have high purity (Xu *et al.*, 2017). This technology uses high-pressures between 100 and 600 MPa and low-to-mild temperatures, preserving compounds structure, since it does not affect covalent bonds (Huang *et al.*, 2017). The large pressure differential created between the interior and the exterior of the cell leads to cell deformation and wall damage, which allows a quick solvent permeation into the cells through the channels generated, with a large rate of dissolution (Xi & Luo, 2016).

The main goal of this work was to analyse the impact of high-pressure, extraction time and ethanol concentration on antioxidant activity, total phenolics, flavonoids, tannins, betalains, carotenoids and anthocyanins extracted from yellow prickly pear peel. The results for each parameter were modelled by Response Surface Methodology (RSM), the optimum extraction conditions were determined, models were validated and results were compared with Soxhlet extraction. Antibacterial activity against *Escherichia coli* and *Listeria innocua* was analysed for selected extracts.

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Methods

Plant material

Prickly pear peels were kindly provided by 'Cactus Extractus Lda' located in Évora/Portugal. The peels were dried by convection at 40 °C until a moisture content of 13 ± 2% (dry basis) what took around 1.5 days of drying, grounded, vacuum packaged and stored at −20 °C.

Extractions

A high-pressure equipment (Hyperbaric 55, Hyperbaric, Burgos, Spain) with a maximum operation pressure of 600 MPa was used. Grounded peel (0.5 g) were added to 20 mL of an ethanol solution (0%, 40% and 80% in water) in plastic bags, which were pressurised at 300 and 600 MPa during 5, 17.5 and 30 min, at room temperature. Control experiments were performed alike but at 0.1 MPa.

Soxhlet extraction was performed using ethanol 40% for 4 h in a Soxhlet apparatus at 115 °C.

All extracts were centrifuged at 18 200 ***g*** for 10 min at 4 °C (Thermo Fisher Scientific, Massachusetts, USA), filtered and frozen at −40 °C until used. Analyses were performed in triplicate using a 96-well microplate and read in a microplate reader (Microplate Spectrophotometric Multiscan Go, Thermo Scientific, USA).

Total phenolics

A modified Folin-Ciocalteu assay was employed. Briefly, 20 μL of sample was added to 100 μL of 1:4 diluted Folin-Ciocalteu reagent reacting for 4 min. Then, 75 μL of Na_2CO_3 solution (100 g L^{-1}) was mixed and after 2 h, the reads were performed at 750 nm. Gallic acid (GA) was used as standard and total phenolics were presented as mg GA Equivalent/g DW.

Total flavonoids

Total flavonoids were quantified using Dowd method. Briefly, 150 μL of 2% of $AlCl_3$ and 150 μL of methanol were added with 150 μL of each sample separately and after 10 min were read at 415 nm. Quercetin (QRC) was used as standard and total flavonoids were expressed as mg QRC Equivalent/g DW.

Total condensed tannins

The vanillin method was used to quantify the tannins (Naczk *et al.*, 2000). The extracts (50 μL) were mixed with 150 μL of vanillin (1% in 7 M H_2SO_4), for 15 min and the reads were performed at 500 nm. Catechin was used as standard and the tannins were presented as mg catechin Equivalent/g DW.

Total betalains

Total betacyanins (BCY) and betaxanthins (BX) content were quantified using the method reported by Stintzing *et al.* (2005) by mixing of 275 μL of extract with 25 μL of McIlvaine buffer (pH 6.5, citrate-phosphate) to obtain absorption values of $0.9 \leq A \leq 1.1$ and read at 480 and 538 nm for BX and BCY respectively. Concentrations were calculated using the Equation 1:

$$C = \frac{A \times \mathrm{MW} \times df \times 1000 \times V}{0.77 \times m \times \varepsilon} \quad (1)$$

where A is the OD difference between samples and blank; MW and ε is the molecular weights and molar extinction coefficients of betanin (BT) (MW = 550 g mol^{-1}; ε = 60 000 L $mol.cm^{-1}$ in water; 538 nm) and indicaxanthin (IN) (MW = 308 g mol^{-1}; ε = 48 000 L $mol.cm^{-1}$ in water; 480 nm) for quantification of BCY and BX respectively; m and V is the mass of residue and the volume of extract; df is the dilution factor and 0.77 is the path length in centimetres for microplate reader. Results were expressed as mg BT Equivalent/g DW for BCY and as mg IN Equivalent/g DW for BX.

Total carotenoids

Total carotenoids (CR) content was determined using the method reported by Wang *et al.* (2008). The absorbance was read at 450 nm, β-carotene (BC) was used as standard and the results were expressed as mg BC Equivalent/g DW.

Antioxidant activity

Antioxidant activity was quantified by DPPH, ABTS and FRAP, according to Alexandre *et al.* (2017a). For DPPH method, 20 μL of sample was mixed with 180 μL of DPPH reagent (150 μM) and the absorbance was measured at 515 nm, 40 min later. For ABTS, a stock solution of $ABTS^{\cdot+}$ was obtained by the reaction of ABTS 7 mM with potassium persulfate 2.45 mM for 24 h in dark, being then diluted to an absorbance of 0.80 ± 0.02 at 734 nm. Then, 200 μL of this diluted solution was added to 20 μL of sample and 6 min later the absorbance was read. Trolox (TR) solutions were used as standard and results were presented as mg TR Equivalent/mg DW.

The FRAP working solution was prepared with 50 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of TPTZ 10 mM (prepared in 40 mM HCl) and 2.5 mL of

ferric chloride (20 mM in water), which was warmed at 37 °C for 10 min. Then, 280 μL of this solution was added to 20 μL of sample, incubated at 37 °C for 30 min and absorbance was measured at 595 nm. Ammonium iron (II) sulfate (AIS) solutions were used as standard and results were presented as mg AIS Equivalent/g DW.

Total extraction yields

The extraction yields were determined based on the relation between the mass of the dried samples and the mass used to do the initial extracts.

Antibacterial activity

Antibacterial activity was tested against *Escherichia coli ATCC 25922* and *Listeria innocua ATCC* 33090 (Liofilchem, Roseto degli Abruzzi (TE), Italy) using the Kirby-Bauer well-diffusion method. The cultures were regenerated at 37 °C for 24 h and the bacterial suspensions were adjusted to 0.5 MacFarland at 625 nm. Plates containing Müller-Hinton agar were inoculated and 6 mm diameter wells were perforated. Aliquots (50 μL) of extract (1 mg μL^{-1}) were dispensed into wells and plates were incubated for 24 h at 37 °C. Ampicillin and water were used as positive and negative controls respectively. The halos formed by inhibition zones surrounding wells were considered as a measurement of the antimicrobial activity.

Experimental design, statistical analysis and models validation

The experimental methodology was performed based on a full 3^3 Box–Behnken design and data were analysed by RSM. The effect of high-pressure, extraction time and ethanol concentration was analysed in all variables. The central point was replicated three times for error assessment. The output results were fitted to a second-order polynomial equation (quadratic model), according to the model in Equation 2.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i \neq j=1}^{k} \beta_{ij} x_i x_j \qquad (2)$$

where Y is the predicted response, β_0 is the intercept coefficient, β_i, β_{ii} and β_{ij} are the linear, quadratic and linear interactive coefficients, respectively, and xi and xj represent the independent variables. The regression coefficients (linear, quadratic and interaction terms) of each polynomial equation were determined using ANOVA. These coefficients were used to generate 3-D surface plots to obtain the relationship between the response and experimental levels of each factor and to determine the optimum extraction conditions. ANOVA analysis was performed for each response variable using the models, where P-values indicated whether the terms were significant ($P < 0.05$) or not. To validate the models, additional extraction trials performed were carried out at the predicted optimal conditions and the experimental data were compared to the values predicted by the regression model. Antibacterial activity was analysed by ANOVA.

Results and discussion

Total phenolics, flavonoids and tannins

In general, high-pressure resulted in an increase of total phenolics of 11%, but the highest extraction yield obtained was 26.30 ± 1.06 mg GA Eq./g DW, for 600 MPa, 17.5 min and 0% of ethanol, which represents an increase of 14% (0.1 MPa) (Table S1). The increase of pressure level and extraction time led to higher phenolic extractions (Figure S1 a). For flavonoids, the highest extraction yield obtained was 2.19 ± 0.04 mg QRC Eq./g DW for 600 MPa, 30 min and 40% of ethanol, resulting in an increase of 35% (0.1 MPa), but it was for an intermedium high-pressure that obtained higher extraction yields (Figure S1 b). Alexandre *et al*. (2017b) studied high-pressure effect on total phenolics extraction from pomegranate and obtained an increase of 12% and 6% when 300 and 600 MPa were used respectively. The main reason for the enhanced extraction of phenolics and flavonoids with pressure increase can be related to the cell wall breakdown. The influx of greater amounts of solvent to the inner membranes can facilitate the extraction of compounds (Xi & Luo, 2016). High-pressure can lead to changes on the conformation and cause protein denaturation that will make phenolic compounds linked with proteins more suitable to be extracted (Fernandes *et al*., 2017).

In all cases, phenolics extraction yield decreased when 80% ethanol was used but were quite similar when 40% of ethanol or water was used. For 300 MPa, 30 min, phenolics content increased/decreased from 21.75 ± 1.64 to 25.28 ± 1.37 and 16.20 ± 1.96 mg GA Eq./g DW, when 0%, 40% and 80% of ethanol was used respectively. Pressure and ethanol concentration effects were significant, but the main effect observed was due to solvent concentration (F values of 326 and 174 for linear and quadratic solvent effects respectively). Also for flavonoids, ethanol concentration had the most significant impact presenting F values of 171 and 68 for linear and quadratic effects respectively (Table 1). In all cases (except for 300 MPa, 30 min), total flavonoids extraction yield decreased only when water was used as a solvent. For 300 MPa, 17.5 min, flavonoids increased from 1.09 ± 0.09 to 1.71 ± 0.08 and 1.89 ± 0.04 mg QRC

Eq./g DW, when 0%, 40% and 80% of ethanol was used respectively. Ćujić *et al.* (2016) reported that hydro-organic solvents are more efficient for the extraction of phenolic compounds than water or pure ethanol. Zhang *et al.* (2017) reported that the solubility of flavonoids is linked to its structure and to the weaker and strong polarities of ethanol and water, respectively, making flavonoids less soluble in water.

Total condensed tannins were not detected.

Total monomeric anthocyanins, betalains and carotenoids

Total anthocyanins were not detected but betalains were quantified regarding yellow-orange betaxanthins since anthocyanins and betalains are mutually excluding compounds (Polturak *et al.*, 2017). The highest extraction yield of betaxanthins was obtained for 300 MPa, 30 min, 40% of ethanol (0.26 ± 0.00 mg IN Eq./g DW), which was 18% higher than control (0.1 MPa), but in general this pressure conduced to an increase of 13%. The betaxanthin concentrations obtained are similar to those reported by Ramírez-Ramos *et al.* (2018). The increase in pressure led to higher extraction yields (Figure S1 c), but it was for ethanol concentration that was verified the highest effect (*F* values of 1452-linear and 1234-quadratic effects), being preferable low concentrations. When 40% of ethanol was used, the yield of betaxanthins increased 14% in relation to extractions performed with water. Melgar *et al.* (2017) reported that betalains are almost water soluble pigments, but they are also slightly soluble in ethanol (Damit *et al.*, 2017). However, Damit *et al.* (2017) reported that a 60% ethanol concentration increased betalains extraction in relation to water or ethanol.

Ethanol concentration was also the variable that had the highest impact (*F* values between 864 and 475) on total carotenoids extraction yield, followed by high-pressure (*F* values between 48 and 6). The highest yield obtained was 0.40 ± 0.00 mg BC Eq./g DW, for 600 MPa, 17.5 min and water, representing an increase of 48% (0.1 MPa). 600 MPa conduced to a higher increase of carotenoids extraction than 300 MPa (17% and 11% respectively), when compared with 0.1 MPa. When an ethanol concentration of 40% was used, the extraction increased 5% compared with water extractions. According to Figure S1 d), the extraction using higher pressures led to higher carotenoid extraction at low ethanol concentrations. Carotenoids are localised in the chloroplast or accumulated in vesicles and plasma and chloroplast membranes limit the rate of mass transfer of carotenoids during extraction processes. However, high-pressure extraction is based on the physical membrane disruption or permeabilisation to increase the rate of mass transfer of carotenoids from the intracellular spaces (Poojary *et al.*, 2016). Strati *et al.* (2015) extracted carotenoids from tomato and it was for higher pressures wherethe highest extraction yields were observed. For 10 min and 700 MPa, the carotenoids extraction increased between 2% and 26% depending on the solvent used. These authors, also reported that denaturation of carotenoid-binding proteins by high-pressure could be involved, facilitating the extraction of carotenoids.

Extraction time was not statistically significant.

Table 1 Analyses of variance for linear, quadratic and crossed effects of pressure, extraction time and ethanol concentration at a significance level of 95% confidence and the determination coefficients of each model

	PC		FL		BX		CR		ABTS		DPPH		FRAP		TY	
ANOVA	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
P(L)	24.07	**0.00**	0.57	0.45	0.68	0.41	48.38	**0.00**	18.42	**0.00**	0.01	0.92	22.34	**0.00**	9.86	**0.00**
P (Q)	0.31	0.58	0.58	0.45	18.49	**0.00**	6.25	**0.01**	5.96	**0.02**	1.89	0.17	37.79	**0.00**	13.39	**0.00**
t (L)	3.21	0.08	2.01	0.16	7.24	**0.01**	0.54	0.47	12.40	**0.00**	4.80	**0.03**	10.72	**0.00**	0.00	0.98
t (Q)	2.10	0.15	1.57	0.21	0.75	0.39	0.18	0.67	1.72	0.19	0.23	0.63	30.67	**0.00**	0.30	0.59
E (L)	325.61	**0.00**	170.60	**0.00**	1452.25	**0.00**	864.68	**0.00**	166.05	**0.00**	242.35	**0.00**	209.46	**0.00**	369.22	**0.00**
E (Q)	173.95	**0.00**	67.76	**0.00**	1234.21	**0.00**	475.44	**0.00**	302.43	**0.00**	128.17	**0.00**	280.84	**0.00**	243.25	**0.00**
P (L) x *t* (L)	1.91	0.17	0.04	0.84	0.77	0.38	5.08	**0.03**	4.33	**0.04**	10.17	**0.00**	12.54	**0.00**	2.56	0.11
P (L) x *E* (L)	9.08	**0.00**	3.41	0.07	86.10	**0.00**	14.26	**0.00**	0.81	0.37	9.72	**0.00**	0.33	0.56	30.90	**0.00**
t (L) x *E* (L)	1.36	0.25	0.76	0.38	0.98	0.33	0.45	0.50	0.17	0.69	0.16	0.69	0.37	0.55	0.96	0.33
R^2	0.875		0.757		0.973		0.947		0.863		0.836		0.885		0.897	
R^2 adjst	0.861		0.730		0.970		0.941		0.848		0.817		0.872		0.886	

ABTS, DPPH and FRAP, antioxidant activity by the ABTS, DPPH and FRAP methods; BX, betaxanthins; CR, carotenoids; *E*, Ethanol percentage (%); FL, flavonoids; L, linear; *P*, Pressure (MPa); PC, total phenolic compounds; Q, quadratic; *t*, time (min); TY, total yields.
The significant coefficients ($P < 0.05$) in each case are written in bold.

Antioxidant activity

Antioxidant activity was significantly affected mainly by ethanol concentration (F values between 166 and 302), followed by high-pressure (F values between 6 and 38) and extraction time, which had the lowest effect but still significant (F values between 5 and 31). In general, higher extraction time and intermediate ethanol concentration allowed higher antioxidant activity. Higher pressures increased antioxidant activity measured by ABTS, but intermediate pressures improved antioxidant activity measured by DPPH and FRAP. The highest antioxidant activities were 21% (ABTS), 19% (DPPH) and 13% (FRAP) higher than control, but in general, high-pressure conduced to an increase between 5% and 18%. Casquete *et al.* (2015) reported that 300 MPa, 10 min and 500 MPa, 3 min resulted in higher quantifications of antioxidant activity (DPPH) in orange (14%) and lemon (25%) peels respectively. The amount of phenolic compounds increased with pressure increase, which in turn led to an increase of antioxidant effects. The reduction of DPPH radical by the peel extracts has been attributed to the presence of phenolic compounds. Their capacity varies from one compound to another, but there might be a synergy between them and/or other constituents that can be present in the extracts (Casquete *et al.*, 2015).

Total extraction yields

Total yields were significantly affected mainly by ethanol concentration (F values were 369-linear and 243-quadratic effects) and high-pressure (F values of 9.86-linear and 13.39-quadratic effects). The highest yield ($48.11 \pm 2.53\%$) was obtained for 300 MPa, 5 min and 40% of ethanol, which represented an increase of 12% (0.1 MPa). Intermediate high-pressure and low ethanolic solutions were better to improve extraction yields (Figure S1 h). In general, the use of high-pressure resulted in an increase in the extraction yields of 6%. Also a 6% increment was obtained when an ethanol concentration of 40% was used, compared with water. In pomegranate peels, Alexandre *et al.* (2017b) reported a yield increase of 6% and 3% when 300 MPa and 600 MPa were used respectively. As mentioned above, the solvent permeability is favoured by the pressure, spending much lower times and consequently more compounds can be extracted improving also total extraction yields.

Models fit and adequacy

The predicted values were in good agreement with the experimental results, since they differed in average <7% indicating that RSM is good and accurate, except for flavonoids and antioxidant activity (DPPH method), where values differ in average 9% and 10% respectively.

Except for flavonoids, the R^2 and R^2_{ajust} were high, between 0.836–0.973 and 0.817–0.970 respectively (Table 1). Consequently, the models showed a high fit and adequacy to experimental data since they explain more than 84% of total variations observed. It was for betaxanthins and carotenoids models that were obtained the highest R^2 (0.973 and 0.947 respectively), meaning that only 2.7% and 5.3% of the experimental values were not explained by the models.

The coefficient of variation was lower than 10%, with few exceptions, expressing a good precision and repeatability of the conducted experiments.

Each model could be expressed by Equation 2 as a function of the independent variables within the region under investigation by applying multiple regression analysis to the experimental data. The regression coefficients were determined and presented in Table S2. The coefficient values obtained were generally low due to the high number of experiences performed and the intercept coefficients (β_0) were statistically significant for all cases ($P < 0.05$).

Correlation matrix

All correlations between antioxidant activity, extraction yields, phenolic compounds and betaxanthins were significant ($P < 0.05$) and changed between 0.53 and 0.95 (Table S3). The strongest correlation was found between the total extraction yields and betaxanthins ($r = 0.95$). Betaxanthins and carotenoids presented the highest correlations with antioxidant activity assays, indicating that most part of antioxidant activity maybe more related with betaxanthins and carotenoids than with phenolic compounds. Cardador-Martinez *et al.*(2011) also studied the correlation between total phenolics and antioxidant activity (DPPH and ABTS) from prickly peels. The authors reported similar significant correlation between the phenolic compounds and DPPH antioxidant activity (0.52), and between DPPH and ABTS antioxidant activities (0.64) but did not study betaxanthins and carotenoids.

Optimum extraction conditions, validation of models and comparison with Soxhlet

The optimum conditions were strongly depended on the parameter analysed. Extraction time was the variable with lower impact in the responses but their optimum values changed between 5 min (flavonoids, betaxanthins, carotenoids, total yields) to 30 min (antioxidant activity). Optimum ethanol concentration changing between 22% and 31% (except for flavonoids,

Table 2 Optimum extraction conditions, predicted optimum values (POV), experimental optimum values (EOV) and Soxhlet extraction results

	PC	FL	BX	CR	ABTS	DPPH	FRAP	TY
P (MPa)	600	496	174	600	600	118	303	294
t (min)	16	5	5	5	30	30	30	5
E (%)	26	60	25	21	31	26	30	22
POV (mg St. Eq./g DW)*	26	1.97	0.238	0.37	14	16	53	45
EOV (mg St. Eq./g DW)*	27 ± 1^{b}	1.81 ± 0.02^{a}	0.236 ± 0.002^{b}	0.32 ± 0.00^{b}	15 ± 0^{b}	17 ± 1^{a}	52 ± 1^{a}	44 ± 1^{a}
Soxhlet (mg St. Eq./g DW)	23 ± 1^{a}	2.33 ± 0.04^{b}	0.109 ± 0.002^{a}	0.28 ± 0.00^{a}	10 ± 0^{a}	14 ± 1^{a}	51 ± 1^{a}	45 ± 0^{a}

ABTS, DPPH and FRAP, antioxidant activity by the ABTS, DPPH and FRAP methods; BX, betaxanthins; CR, carotenoids; FL, flavonoids; PC, total phenolic compounds; TY, total yields.

Different letters indicate significant differences ($P < 0.05$) between Soxhlet and EOV (mean ± standard deviation; $n = 3$).

*mg standard equivalent/g DW) except for total yields, which are presented in %.

60%) and the optimum pressure was the variable that changed more between all parameters (Table 2).

The optimum predicted extraction values for each parameter were close to the results obtained experimentally under optimum conditions defined by each model, validating the models. For betaxanthins, total extraction yields and antioxidant activity (FRAP) models, results only differ 1%, 2% and 3% respectively.

When compared with Soxhlet, optimised conditions increased the extraction yields or at least were similar.

Antibacterial activity

Escherichia coli was more resistant than *Listeria innocua*, but both bacteria were inhibited by the extracts. Inhibition halos obtained for *Listeria innocua* vary between 17 and 21 mm, while for *Escherichia coli* changed between 8 and 10 mm. In both cases, it was for the extract obtained at 496 MPa, 5 min and 60% of ethanol (optimum extraction conditions for total flavonoids) that was obtained the highest inhibition halos being statistically different from the obtained with the other extracts. Similar results were obtained by Casquete *et al*. (2015) when studied the effect of high-pressure on antimicrobial activity of citrus peels.

Conclusions

The three variables studied significantly influenced the extraction of total compounds, independently and interactively. Ethanol concentration was the variable that showed the highest effect on extraction yields, followed by high-pressure and then extraction time. In general, high-pressure extraction increased extraction yields between 6% and 17% and the high correlation of mathematical models indicated that the quadratic polynomial models could be employed to optimise extraction conditions. The fitting and adequacy of models were high since the R^2 values obtained were high, except for total flavonoids. Moreover, the predicted values were very close to the experimental results indicating a good adequacy of models. The optimum extraction conditions were established, predicted and experimental results were close, differing <10%, however, optimum conditions are dependent of the compound family to be extracted. The selected extracts showed antibacterial activity against both bacteria. The optimisations obtained in this work make high-pressure assisted extraction, a promising process to improve extraction of bioactives from prickly pear peels.

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Conflict statements

The authors declare no competing financial interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Response surface plots using 80% ethanol: (a) phenolics, (b) flavonoids, (c) betaxanthins, (h) total yields or 40% ethanol: (d) carotenoids, (e) ABTS, (f) DPPH, (g) FRAP methods.

Table S1. Experimental results for the quantification of the bioactive compounds from prickly pear peels and average variation between predicted and experimental results.

Table S2. Regression coefficients of the second-order polynomial regression equation. In regression coefficients, 0 means constant, 1 pressure, 2 extraction time and 3 ethanol concentration.

Table S3. Coefficient correlation matrix between the responses of all dependent variables.